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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/560,595	03/16/2006	Koichiro Kano	8062-1033	7278

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EXAMINER	
POPA, ILEANA	
ART UNIT	PAPER NUMBER
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/560,595	KANO, KOICHIRO	
	Examiner	Art Unit	
	ILEANA POPA	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 February 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *15-34* is/are pending in the application.

4a) Of the above claim(s) *20-22,26-28,33 and 34* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *15-19,23-25 and 29-32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *13 December 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☒ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *See Continuation Sheet*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Attachment(s) 3). Information Disclosure Statement(s) (PTO/SB/08), Paper No(s)/Mail Date :02/28/2007; 10/02/2006; 12/13/2005.

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of the invention of Group II, drawn to a method of transdifferentiating adipocytes into myoblasts in the reply filed on 02/29/2008 is acknowledged. The traversal is on the ground(s) that no one, including Park et al., in the past has shown success in starting from a fat tissue sample. As discussed in the present specification, adult stem cells derived from bone marrow stromal have been used as donor cells in the past, but there are disadvantages to doing so. Park et al. appreciated that the cells present in the floating low-density layer of human bone marrow are mainly adipocytes and pre-adipocytes. Single adipocytes are present together with adipocytes associated in conglomerates with fibroblastic cells (see, e.g., the discussion of the Park et al. experiments on page 553). The various stromal cells present in bone marrow tissue form clusters with adipocytes, and these clusters are likely to exist in the "cells contained in a suspended fraction after centrifugation of the bone marrow samples", which is the "adipocyte" of Park et al. The prior art, e.g., JP 2000083656 of the IDS filed December 13, 2005, shows that in order to obtain a unilocular adipocyte, collagenase processing is needed, which includes multiple rounds of centrifugation and a filtration mesh of about 250μm. Park et al., however, fail to disclose or suggest any collagenase treatment and filtration and do not disclose checking the suspended fraction obtained to see if it is composed solely of adipocytes. However, collagenase treatment and filtration are necessary, i.e., due to the tissue

structure of bone marrow, to obtain a single fraction of adipocyte. Collagenase treatment and filtration are essential for the following reasons. The structure of bone marrow tissue includes reticular tissue that forms stromata and the hematopoietic cells fill the mesh of it. Reticular tissue consists of reticular cells and reticular fibers, thereby forming a microenvironment for induction of differentiation and modulation of hematopoietic cells. Reticular cells include fibroblast, preadipocyte, cells surrounding blood vessels, smooth muscle cells, bone marrow stern cells, and so on, which produce collagens. It is known that some of the reticular cells, pre-adipocytes, differentiate into adipocytes and fill the gap within the pith cavity when hematopoiesis is inactive. Thus, there must be stem cells which can differentiate into pre-adipocytes and adipocytes. These cells within the reticular tissue cannot be distinguished from one another because they all have similar shapes. Consequently, in order to isolate the adipocyte in the reticular tissue of bone marrow, it is necessary to fractionate the cells by digesting the reticular fibers with collagenase. The isolation of the cells requires the follow up filtration step to remove undigested tissue. Centrifugation is used to force the adipocytes more to the upper layer fraction because lipid droplets abundant in the cytoplasm cause buoyancy while the other cells precipitate. Thus, the pure adipocyte can be isolated. Ceiling culture, as seen in the present application, enables floating adipocytes exclusively to be cultured. One skilled in the art at the time the invention was made was well aware that if the enzyme digestion and filtration process is not followed, the adhesive cells recovered will contain various types of cells from the bone marrow tissue. Obtaining of fibroblast-like adipocytes from the cell suspension containing only

mature adipocytes is allegedly carried in Park et al. in sidewell plates. One skilled in the art would be aware that it is impossible for the suspended cells to attach to the bottom of the wells because they remain suspended within the upper portion of the culture and medium due to the large amount of lipid droplets in cytoplasm. In the present invention, the inventors performed ceiling culture to obtain the adipocytes. The adipocytes attach themselves to the surface of the dish allowing them to display proliferative capacity. Park et al., however, do not take this measure. Accordingly, the cell cluster containing adipocytes and bone marrow stromal cells, when cultured in six well culture dishes, is going to lead to fibroblasts falling off and there is doubt, therefore, as to the conclusions of Park et al. These fibroblasts from bone marrow stromal cells could be ones which are subjected to later processing by Park et al. In order to obtain the fibroblast-like cells, Park et al. use a culture medium in which the fraction was induced to differentiate into an adipocyte. However, a culture medium for inducing differentiation usually has the potential of maintaining the function of the adipocyte and suppressing dedifferentiation. There is a contradiction here in using a culture medium to induce differentiation in order to dedifferentiate the adipocyte. Yet it makes sense if the starting point consists of pluripotent stem cells, which will differentiate into adipocytes. One skilled in the art would therefore doubt the disclosure of Park et al. as confirmation of adipocytes isolation from bone marrow stromal cells. There is no disclosure that the pre-adipocyte cell line in PARK expresses an early marker of osteogenesis, myogenesis or adipogenesis. One skilled in the art has read reports in a number of publications that, when using conventional pre-adipocyte lines, the early differentiation marker genes for

adipogenesis are expressed only after induction of differentiation, but not before induction. The absence of any mention of the early marker genes in Park et al. confirms that the identity of their cells remains unknown. It is quite likely that the cells were originated from the bone marrow stromal cells, and if that is the case, it is not at all surprising that their adipocyte derived cells were induced to differentiate into adipocyte by the culture medium for induction of differentiation into adipocyte. Bone marrow stromal cell populations contain pluripotent stem cells. Park et al. fails to teach the dedifferentiation of mature adipocytes derived from bone marrow followed by their transdifferentiation. Park et al. tried to prove that the cells obtained were derived from adipocytes by using anti-AP2 antibody and Oil red O staining. In fact, differentiation to adipocyte can be confirmed by assessing the expression of AP2 gene that is known to be a late marker gene of the differentiation to adipocyte or by performing Oil red O staining of lipid adipocytes. The cells obtained by Park et al. expressed those markers because they had been cultured in a culture medium which induces the differentiation to adipocyte. That is, these cells were the cells which had already been differentiated into adipocyte and possessed the function of adipocytes. In these differentiated cells, the expression of genes and proteins which are specifically expressed in other types of cells (bone, muscle and chondrocyte, for example) is suppressed. In order to differentiate them into another cell type, the function of the adipocyte must be turned off by dedifferentiation. However, there appears to be no such measure taken for the cells obtained by Park et al. The cells obtained must be different from the pre-adipocyte strain of the claimed invention. Indeed, the cells are most likely the cells derived from

multilocular adipocytes that have been induced by differentiation of bone marrow stromal cells that were contaminated during isolation. Thus, for the reasons discussed above Park et al. cannot teach transdifferentiating adipocytes into cells with an osteogenic phenotype exhibiting the markers of osteogenesis, and there is no basis for a determination of lack of unity. Applicant directs Examiner's attention to the paper included in the appendix, i.e., Matsumoto et al., "Mature Adipocyte-Derived Dedifferentiated Fat Cells Exhibit Multilineage Potential", which shows that the preadipocyte cell line of the present invention is a novel cell line which Applicant isolated from mature adipocytes. For all these reasons, Applicant requests the withdrawal of the restriction requirement.

Applicant's arguments have been considered however, they were not found persuasive for the following reasons:

The instant claims are drawn to a method of transdifferentiating a preadipocyte cell line obtained by dedifferentiating mature adipocytes into cells expressing markers of osteogenesis, myogenesis, or adipogenesis. Park et al. teach isolating the floating fat layer obtained after the centrifugation of bone marrow cells (i.e., adipocytes and not bone marrow stromal cells, as Applicant argues), culturing them in adipocyte differentiation medium (i.e., obtaining mature adipocytes), cloning the cells, inducing their dedifferentiation, and examining the dedifferentiated cells for their osteogenic potential; examination for osteogenic potential is done by culturing the dedifferentiated cells osteogenic medium and observing osteogenic markers such as alkaline phosphatase and osteocalcin (Abstract, p. 550, columns 1 and 2, p. 553, column 1, first

full paragraph, p. 553, column 2). Therefore, the argument that there is no disclosure that the pre-adipocyte cell line in Park et al. expresses an early marker of osteogenesis, myogenesis or adipogenesis is inaccurate. Applicant's argument that his method of obtaining the mature adipocyte is different from the method of Park et al. is irrelevant. The claim does not require a specific method of obtaining the mature adipocytes to be dedifferentiated. And even if it did, a mature adipocyte is a mature adipocyte, regardless of the method of obtaining it. It is concluded that Park et al. teach all the limitations recited in claim 1, and therefore, the requirement is still deemed proper.

However, because a search and examination for the invention of Group II yielded results relevant for the inventions of Groups I, **the restriction requirement between the inventions of Groups I and II, as set forth in the Office action mailed on 01/02/2008 is hereby withdrawn as to any claim that requires all the limitations of an allowable claim.** In view of the above noted withdrawal of the restriction requirement, Applicant is advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application.

Once a restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

The restriction between the inventions of Groups I/II and Groups III-V is maintained and made FINAL.

Claims 1-14 have been cancelled. Claims 20-22, 26-28, 33, and 34 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Claims 15-19, 23-25, and 29-32 are under examination.

Information Disclosure Statement

2. The references disclosed on the IDS form of 12/13/2005 have been lined through because Applicant did not provide the documents.

Priority

3. It is acknowledged that a certified foreign priority paper has been received. However, an English translation has not been provided. Correction is required.

Should Applicants provide a certified translation of their foreign priority document to overcome the prior art rejection, Applicants should indicate whether the priority application is identical to the instant application, or if the priority application contains additional disclosure. If there is additional disclosure, a brief summary should be provided. Applicants should also indicate where support for each of the claim limitations (for the independent claims) can be found in the translated priority document by page and line number. If support is not found *in ipsis verbis*, clarification on the record may be helpful to the examination process.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 15, 18, 23, and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Park et al. (Bone, 1999, 24: 549-554, Applicant's IDS), as evidenced by Lecoeur et al. (Biomaterials, 1997, 18: 989-993, Abstract).

Park et al. teach isolating, cultivating, and cloning mature adipocytes from human bone marrow; and the cloned mature adipocytes are further dedifferentiated to fibroblast-like fat cells (i.e., pre-adipocytes), wherein the pre-adipocytes express alkaline phosphatase, i.e., an early marker of osteogenesis (see Lecoeur et al., Abstract) (claim 15); Park et al. teach further transdifferentiating their pre-adipocytes into osteoblasts (claims 18, 23, and 24) (Abstract, p. 550, columns 1 and 2, p. 553, column 1, first full paragraph and column 2). Since Park et al. teach all claim limitations, the claimed invention is anticipated by the above-cited art.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 15-18, 23, 24, 29, and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Park et al. taken with Lecoeur et al., in view of Sugihara et al. (Differentiation, 1986, 31: 42-49, Applicant's IDS).

The teachings of Park et al. and Lecoeur et al. are applied as above for claims 15, 18, 23, and 24. Park et al. do not teach deriving their pre-adipocytes from the dedifferentiation of mature adipocytes isolated from subcutaneous fat tissue (claim 17). However, at the time the invention was made, deriving mature adipocytes from subcutaneous fat tissue and dedifferentiating them to fibroblast-like fat cells (i.e., pre-adipocytes) were both taught by the prior art. For example, Sugihara et al. teach a method of obtaining mature adipocytes from abdominal fat tissue, the method comprising chopping the tissue into small pieces, subjecting the chopped tissue to collagenase digestion followed by filtration and centrifugation, isolating the floating fat cells, followed by subjecting the isolated fat cells to "ceiling culture" to obtain pre-adipocytes (Abstract, p. 42, column 2, p. 44, column 1, second and third paragraphs, p. 45, column 1, p. 46, column 2). It would have been obvious tone of skill in the art, at the time the invention was made, to substitute the pre-adipocytes of Park et al. with those of Sugihara et al. to achieve the predictable result of transdifferentiating them into osteoblasts. Claim 14 recites that the preadipocyte cell line is FERM BP-0864, wherein FERM BP-0864 cell line is obtained by the method of Sugihara et al. (see the instant specification, p. 8, 22, and 23). It is noted that Applicant did not provide any evidence that FERM BP-0864 cell line has unique properties as compared to other cell lines obtained by using the same method, i.e., the method of Sugihara et al. Absent

evidence of unexpected results, it is generally not inventive to use the FERM BP-0864 cell line versus similar cell lines obtained by using the same method. Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

8. Claims 15-19, 23-25, and 29-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Park et al. taken with both Lecoeur et al. and Sugihara et al., in further view of each Ross et al. (Science, 2000, 289: 950-953), Bennett et al. (J Biol Chem, June 7, 2002, 277: 30998-31004), and Rando et al. (J Cell Biol, 1994, 125: 1275-1287).

Park et al., Lecoeur et al., and Sugihara et al. do not teach transdifferentiation to myoblasts (claims 19, 25, 31, and 32). However, at the time the invention was made, the prior art suggested that preadipocytes have the capability to transdifferentiate into myocytes. For example, Ross et al. teach that adipocytes and myocytes originate from the same precursor and that signaling by Wnt10b is required for commitment to the myocyte lineage; they also teach that inhibition of Wnt10b signaling in preadipocytes and myoblasts induces adipogenesis (Abstract, p. 952, columns 2 and 3). Bennett et al. teach that the Wnt10b receptors are highly expressed in preadipocytes and that inhibition of Wnt10b signaling leads to adipogenesis (Abstract, p. 30999, column 1, first paragraph). Based on these teachings, one of skill in the art would have known that treating preadipocytes with a myoblast differentiation medium comprising Wnt10b would result in their transdifferentiation to myoblasts. It would have been obvious to one of skill in the art, at the time the invention was made, to modify the method of Park et al.,

Lecoeur et al., and Sugihara et al. by transdifferentiating their preadipocytes to myoblasts, with a reasonable expectation of success. The motivation to do so is provided by Rando et al., who teach that myoblasts grown *in vitro* can regenerate muscle fibers when transplantated into a subject in need of treatment (Abstract, p. 1275, column 2, p. 1276, column 1). One of skill in the art would have been expected to have a reasonable expectation of success in doing so because the art teaches that preadipocytes express receptors for factors necessary for myoblast lineage commitment. Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

9. No claim is allowed. No claim is free of prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILEANA POPA whose telephone number is (571)272-5546. The examiner can normally be reached on 9:00 am-5:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Ileana Popa, PhD

/Joseph T. Woitach/

Supervisory Patent Examiner, Art Unit 1633